Proposed Synopsis on

ANTIDIABETIC ACTIVITY AND DIABETIC COMPLICATION PROTECTIVE EFFECT OF BIOACTIVE FRACTIONS FROM INDIGENOUS MEDICINAL PLANTS

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SECTION 01

Introduction:

Diabetes Mellitus is one of the common metabolic disorders with micro and macro vascular complications that results in significant morbidity and mortality. It is considered as one of the five leading cause of death in the world. A worldwide prevalence estimated to between 1% and 5%. Diabetes Mellitus leads to abnormalities in carbohydrate, protein and lipid metabolism and increases the risk of developing atherosclerotic arterial disease by two- to six-fold. Serum total cholesterol, triglycerides and LDL levels are seen tobe increased in diabetes. Diabetes mellitus also disturbs the liver function, due to which the levels of SGOT and SGPT are increased in the blood; beside this nephropathy is a major problem in Diabetes Mellitus. Prior to invention of Insulin this disorder was managed principally by the traditional practices by using medicinal plants. Even after insulin in clinical use, it is not possible to complete cur of Diabetes Mellitus or prevent Diabetes related complications.

In the treatment of Diabetes Mellitus non-pharmacological measures (diet, exercise, and weight loss) remains a critical component of therapy. Along with this Dietary management includes the use of traditional medicines that are mainly derived from plants. Even now approximately 50-80% of the world population is almost entirely dependent upon traditional medicines. There are numerous traditional medicinal plants which reported to have hypoglycemic properties such as *Allium Sativum* (Garlic), *Azadirachta Indica* (Neem), *Ocimum Santum* (Tulsi).

Given a reasonable likelihood that medicinal plants with a long history of human use will ultimately yield novel drug prototypes, systematic and intensive search in plants for new drugs to treat diabetes mellitus seem to be of great utility. This approach seems likely to increase the chances for discovering new drugs for the management of diabetes mellitus. Out of the two types of diabetes, the incidence of non-insulin dependent diabetes mellitus (NIDDM) is much higher than the insulin dependent diabetes mellitus (IDDM).

In view of above present study is envisaged to screen some medicinal plant extracts for its effectiveness in Diabetes Mellitus along with its protective effects against Diabetic complications and is aimed for, ‘Antidiabetic activity and diabetic complication protective effect of bioactive fractions from indigenous medicinal plants.’

SECTION 02

REVIEW OF LITERATURE AND DEVELOPMENT IN THE SUBJECT (PREVIOUS WORK DONE IN THE RELEVANT AREA)

1. Archana Gautam et al, (2010) Described various guidelines for identification, evaluation and standardization of herbal drugs. Identification involves the morphology, microscopy parameter of plants, evaluation and standardization of herbal drugs includes physical, chemical and biological parameters. These parameters are crucial for standardization of herbal drugs
2. Sasidharan S, Chen Y, Saravanan D et al (2011) Suggested that extraction and isolation of bioactive compounds from medicinal plants provide unlimited opportunities for new drug leads because of availability of different medicinal properties. They focused on analytical methodologies and its key challenges.
3. S A survase et al, (2011) in this study revealed that the local people of Marathwada region were using 50 species of medicinally important plant belonging to 30 families. Then observation here also shows that traditional medicine plays a significant role in the life of local people.
4. Dnayneshwar P Ghorband et al, (2011) in this study ethnomedicine survey was carried out in Kinwat forest region of Nanded district in Maharashtra. The tribes are mainly focused upon the forest flora for curing the ailments and disease. A total of 25 ethnomedicines have been recorded here which are used in treatment of different diseases. some of the plants listed here are used as antidiabetic but clinically it is not proved so, we can select for the investigation of those plant.
5. Z.D. Kifle et al, (2022) Different medicinal plant parts were used experimentally for antidiabetic effects in Ethiopia. Among these, leaves (69%) were the most commonly investigated medicinal plant parts followed by roots (14%) and seeds (7%). Most of the investigations were completed with hydro-methanolic extracts to obtain a higher percentage of yield. his review gives collective evidence on the potential antidiabetic activities of medicinal plants in Ethiopia. Moreover, further studies are recommended to substantiate the use of these medicinal plants as an antidiabetic agent.

6. G. Brusotti et al, (2013) the aim of this review is to address natural-products chemists to the choice of the best methodologies, which include the combination of extraction/sample preparation tools and analytical techniques, for isolating and characterizing bioactive secondary metabolites from plants, as potential lead compounds in drug discovery process.

7. Monday Michael Onakpa (2013) Suggested that members of *Abelmoschus* genus have been reported to exhibit diverse medicinal properties ranging from antidiabetic, antimicrobial, anticancer, analgesic, antioxidant and ant plasmodial activities. one of such genus plant have been selected for antidiabetic activity.

8. Tabinda boota et al, (2018) The pharmacology studies demonstrated that its active compounds possess broad range of pharmacological uses, especially in cytotoxic, antioxidant, anti-inflammatory, sedative and antimicrobial activities. so, Illicium anisatum can be used for proposed research work.

9. Karthikeyan M et al (2016) The animal models and in vitro techniques are essentials for developing a new drug for the treatment diabetes. All reported models and advanced techniques have been mentioned which are used for selected medicinal plant extracts and fractions.

Extensive literature survey reveals numbers of medicinal plants are claimed in traditional system for its effectiveness in Diabetes Mellitus or so many plants just evaluated for its anti-hyperglycemic activity but not for their effect on Diabetic complications. Many plants just evaluated for their preliminary activity but not for their extensive screening. Accordingly for present study following medicinal plants are shortlisted for further literature survey so as to select two or three medicinal plants for study.

1. *Abelmoschus ficulnesus (malvaceae)*
2. *Sapindus mukoroossi* (Sapindaceae)
3. *Cinnamonum glandulifeum (Lauraceae)*
4. *Morinda pubescens (Rubiaceae)*
5. *Illicium anisatum (Mangoliaceae)*
6. *Calotropis procera* (*Asclepiadaceae*)
7. *Ferula narthex (Apiaceae)*

and if required any more plant may be selected for the study other than above listed plants.

SECTION 03

OBJECTIVES OF RESEARCH

In this modern era for primary health care, most of the world populations still uses herbal medicine because of its better cultural acceptability, better compatibility with the human body, and lesser side effects. it is estimated that 3% of the world's population have diabetes and the prevalence is expected to double by the year 2025 to 6.3%.

Present study is aimed for - 'Antidiabetic activity and diabetic complication protective effect of bioactive fraction from indigenous medicinal plants.' with objectives

i) Identification, collection and authentication of medicinal plants
ii) Extraction, phytochemical investigation and standardization of medicinal plants.
iii) Safe dose calculation
iv) Evaluation of biological activity of fractions selected for antidiabetic activity and diabetic complications protective effect.
v) Compilation of data and interpretations of data.

SECTION 04

METHODOLOGY TO BE ADDOPTED

Plant Material:

Selected plant parts will be collected from local known sources and get authenticated from expert Botanist of the region.

Processing of Plant material:

Collected crude drug will be subjected to processing such as drying, grinding, sieving etc as per official standards.

Standardization of crude drugs:

It includes estimation for extractive value, acid & water-soluble ash, LOD, presence of foreign matter, heavy metal content, microbial contamination etc.

Pharmacognostic Evaluation of plant material:

Parts of selected plants will be subjected to Pharmacognostic evaluation viz;

a) Morphological Evaluation
b) Microscopical evaluation and powder characteristics

Extraction of Plant material:

Powder form of selected individual plant material will be subjected to extraction. The choice of solvent for extraction and extraction methods will be decided according to chemical constituents of selected plants part and nature of chemical constituents.

Standardization of extracts:

Phytochemical examinations will be carried out for all the extracts as per the standard methods. Qualitative analysis will be carried out for detection of phytoconstituents and later will be confirmed by using suitable chromatographic technique. Total phenolic content and Total flavonoid content will be determined

Bioactivity guided fractionation of extracts

Invitro assay-

i) α amylase inhibitor

A total of 500 µl of test samples and standard drug will be added to phosphate buffer (pH 6.9) containing α-amylase solution and incubate. Then 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) will be added to each tube and incubate. The reaction will stop with 3, 5 dinitrosalicylic acid colour reagent. Then will incubate in a boiling water bath for 5 min, cooled to room temperature. Dilute with 10 ml distilled water and absorbance at 540 nm. Control represents 100% enzyme activity and were conducted in similar way by placing extract with vehicle.

ii) Antioxidant activity

In vitro antioxidant activities for the extracts will be carried out using the following methods

a. DPPH assay

b. Hydrogen peroxide scavenging assay

c. Nitric oxide scavenging assay

d. Ferric chloride scavenging assay

Any two methods from above will be used for antioxidant activity.

Standardization of fraction

Quantative analysis, Qualitative analysis and TLC fingerprinting will be carried out.

Pharmacological Screening of plant extracts and fraction:

Selected plant's part extracts and or fraction either individually or in combination will be evaluated for-

i. **Safe dose calculation:**

Safe dose calculation of plant extracts for the purpose of use of extracts for efficacy evaluation will be carried out as per OECD Guidelines for 'Acute Toxicity study: Class Method (423)'.

ii. **Anti-hyperglycemic**

Antihyperglycemic evaluation of plants part extracts will be carried out in alloxan induced/ streptozotocin induced diabetic animal model as per reference standard methods.

iii. **Diabetic complication protective activity**

Estimation for diabetic complications protective activity against nephropathy or hepatic complications or neuropathy will be carried out in diabetes induced animal model by biochemical parameters like SGPT, SGOT, bilirubin, creatinine, total protein in serum and urine, etc. Gross pathological findings will be also carried out.

Statistical analysis of data and its presentation:

- Data collected that for Pharmacognostic evaluation of selected plants part will be presented in tabular form and microscopic observation will be presented as figures of TS & for powder characteristics.
- Qualitative evaluation of plant extracts for the content of phytoconstituents will be presented as either present (+ve) or absent (-ve) and presence of phytoconstituents will be confirmed by TLC by comparing with Rf value of standard.
- Safe dose will be calculated as 1/10 dose of LD_{50} value of plant extracts.
- The assessment of Antihyperglycemic activity will be observed through decreases in blood glucose level relative to their initial values. (96 h past alloxan streptozotocin values).
- Observations of diabetic complications protective activity of plant extracts will be made on the basis of biochemical parameters like SGPT, SGOT, bilirubin, creatinine, total protein in serum and urine, etc. All data will be compared with control and positive control group for the level of significance.
- All the data related to efficacy of extracts of selected plants part will be subjected to statistical analysis like student's t test, ANOVA etc. to find level of significance between

extracts and between extract to control/positive control data. Such data will also be presented as chart diagram.

SECTION 05

IMPORTANCE OF STUDY/ SOCIETY APPLICATION

Herbal medicine, as a major part of traditional medicine, has been used in medical practice since antiquity and is a common element of ayurvedic, homeopathic and naturopathic medicine. World Health Organization (WHO) notes that 74% of the plant derived medicines are used in modern medicine, in a way that their traditional modern application directly correlates with their traditional use as herbal medicines by native cultures. WHO estimates that 65% of the world's population uses traditional medicines as their primary form of health care and about 85% of traditional medicines involve the use of herbal preparations. The global market of herbal drugs at present is approximately $ 600 billion.

According to WHO, it is estimated that 3% of the world's population have diabetes and the prevalence is expected to double by the year 2025 to 6.3%. Despite the availability of a wide range of drugs, the relief offered by them is mainly symptomatic and short lived. Moreover, these drugs produce side effects. Management of diabetes without side effects is still challenge to the medical community. There comes the importance of Natural remedies. The current accepted modern medicine or allopathy has gradually developed over the years by scientific and observational efforts of scientists. However, the basis of its development remains rooted in traditional medicine and therapies. Herbal medicines are being used by nearly about 80% of the world population, primarily in developing countries for primary health care. Assessing the current status of health care system in adequacies of synthetic drugs is likely to be more glaring in the coming years. It has been reported that there has been an alarming increase in number of diseases and disorders caused by synthetic drugs prompting a switch over to traditional herbal medicine (Rana., 2008).

India has a rich cultural heritage of traditional medicines. The crude drugs being always available easily in abundance, comparatively cheaper, with negligible side effects and have frequently been prescribed to patients of all age groups. The multiple therapeutic action and uses of these drugs are sufficiently described in classical literature on indigenous medicines in many medicinal plant books. Diabetes is a disease that does not have the boundaries of age, race and gender. The

availability of effective medications is not withstanding with present challenges and the prevalence of diabetes is continuously increasing with time. Moreover, the complication and side effects of these drugs are also quite disturbing. Hence there is an increasing demand for use of traditional herbal drug. As existing therapies for the treatment of the diabetes are not complete and satisfactory due to major side effects at higher doses and prolonged treatment, present scenario necessitate new treatment. Selected plant and its part leaves is less explored but is reported to possess important phytoconstituents of wide range which will be useful in diabetes and its complication. Such constituents can be separated, fractionated and evaluated using modern tools for spectral, pharmacological and toxicological profile. Present project may help to explore novel constituents for the mentioned pathologies which could be more effective as well as associated with lesser side effect which will be beneficial for the society. With this regards present project is undertaken with objectives to identify and characterize bioactive component present in the selected medicinal plant extract and to evaluate its antidiabetic and diabetic complication activity.

SECTION 06

PROPOSED WORK PLAN

1. Literature survey
2. Collection, Identification and authentication of selected plant material
3. Processing of plant material
4. Standardization of crude drug
    i) Extractive Value & Ash content
    ii) Acid & Water soluble ash
    iii) Foreign matter
    iv) LOD
    v) Heavy Metal Content
    vi) Microbial Contamination
5. Pharmacognostic evaluation of crude drug
6. Extraction of selected plant materials-
    a) Selection of Extraction method
    b) Selection of Solvent
7. Standardization of plant extracts

a) Qualitative analysis / Identification test
b) TLC fingerprinting
c) Quantative analysis of extract for
   i) Total phenolic content
   ii) Total flavonoids content

8. Bioactivity guided fractionation of extracts

   Invitro assay-

   i) α amylase inhibitor
   ii) Antioxidant activity

9. Standardization of fraction
10. Pharmacological Screening of selected plant extracts/ fraction for-
    i) Safe dose calculation
    ii) Anti-diabetic and Diabetic Complication Protective Activity
11. Statistical analysis of data and its presentation.

YEAR WISE PLAN OF WORK AND TARGET TO ACHIEVE

Present study is designed in such a way that it will come to a conclusive outcome within 03 years from the date of RRC. The year wise plan includes**.**

YEAR 01

Literature survey, Plant Collection and preliminary screening, Morphological and Microscopic evaluation, Drying, Powdering, Physical evaluation of powdered drug.

YEAR 02

Extraction of the Plant material by suitable method of extraction, phytochemical evaluation of extracts/ fractions by different qualitative and quantitative methods of evaluation (as per WHO guidelines). *In vitro* Pharmacological screening of different extracts for anti -diabetic activity

YEAR 03

In vivo screening of anti-diabetic activity of different extracts on suitable animal model and by evaluating various parameters, characterisation of active components present in the extract which will show maximum promising anti-diabetic effect, Compilation, interpretation of data and thesis writing.

SECTION 07

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